IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

P#27

In re application of:			
	SPRINGER *et al.*	Art Unit:	1644
Appl. No.	08/474,388	Examiner:	Gambel, P.
Filed:	June 7, 1995	Atty. Docket:	1011.004000D/SLF/RCM
For:	**ICAM-1 Preparations**		

Declaration of Robert Rothlein Under 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Robert Rothlein, a citizen of the United States, do hereby declare and say:

1. I am presently employed by Boehringer Ingelheim Pharmaceuticals, Inc. [hereinafter "Boehringer Ingelheim"], 900 Ridgebury Road, Ridgefield, CT 06877-0368, as Director, Scientific Research and Development and Business Support, and a Highly Distinguished Scientist. Boehringer Ingelheim is the licensee of the above-identified application.

2. I hold the degree of Doctor of Philosophy in Immunology. My credentials are set forth in my Curriculum Vitae, which is attached hereto as Exhibit A.

3. I have read and understood the specification of the above-identified application (attached as Exhibit B) as well as the Office Action mailed on August 4, 1999 (attached as Exhibit C) [hereinafter "Office Action"], and the Advisory Action mailed on February 18, 2000 (attached as Exhibit D).

4. I have read and understood the content of Tomassini, thesis 8624033 (1986) [hereinafter "the Tomassini thesis"] (attached as Exhibit E); and Tomassini *et al.*, *J. Virol. 58*:290-295 (1986) [hereinafter "the Tomassini article"] (attached as Exhibit F).

5. On information and belief, the pending claims in the above-captioned application recite, *inter alia*, a purified or isolated ICAM-1 substantially free of natural contaminants derived from human cells or tissues and capable of binding to LFA-1, Mac-1, or p150,95.

6. I note that the Examiner has rejected the pending claims under 35 U.S.C. § 102(b) over the Tomassini thesis or the Tomassini article. In making this rejection, the Examiner concluded that

> [Tomassini *et al.*] teach 400-fold immunoaffinity purified 90 kDa HRRP (ICAM-1). The cited document is silent as to whether the 90 kDa HRRP product (ICAM-1) binds to a member of the LFA-1 family, but would inherently have this property as it would comprise the same binding site residues as the ICAM-1 products encompassed by the instant claim language.

(Office Action at 4.) I believe this conclusion is in error given the results disclosed in the Tomassini thesis and Tomassini article. Specifically, in the Tomassini thesis and article, the authors indicate that "[r]epeated attempts to use radiolabeled HRV [human rhinovirus] in place of receptor antibody in the RIA gave inconclusive results owing to poor virus binding." (Tomassini thesis at 44, lines 9-12; and Tomassini article at 292, col. 2, lines 18-21.) The authors further

indicate that "it is quite tempting to speculate that a pentamer of the 90-kDa receptor protein is needed for a functional receptor complex. This would correlate well with the 440-kDa receptor peak obtained by gel filtration and the inability to isolate a 90-kDa receptor protein capable of binding virus." (Tomassini thesis at 116, line 22, to 117, line 1; and Tomassini article at 295, col. 1, lines 20-25.) These statements indicate that Tomassini's purified HRRP (ICAM-1) receptor preparation is not able to bind HRV. The most reasonable scientific explanation is that the purification procedures taught in the Tomassini thesis and Tomassini article disrupt or denature the HRRP receptor structure such that HRV binding is eliminated. Since the binding sites for HRV and LFA-1 overlap, one of ordinary skill in the art would expect that any disruption in structure from the purification procedure leading to the elimination of HRV binding, would also reduce or eliminate LFA-1 binding. Therefore, I do not believe that the HRRP preparation of Tomassini exhibits the ability to bind ligands, in contrast to the HRRP preparation recited in the pending claims, which does.

7. The Examiner supports the anticipation rejection by stating that "poor binding of radio labeled HRV is more likely to be attributable to chemical denaturation and subsequent disruption of the binding sites on the virus (not HRRP or ICAM-1) by radio labeling protocols." (Office Action at 4.) However, I note that the Tomassini thesis and article teach the inhibition of radio labeled HRV binding by polyclonal rabbit antibodies to the 90 kDa protein in membrane binding and cell

protection assays. (Tomassini thesis at 65, lines 1-7, Figure 10 and Table 4; and Tomassini article at 293, col. 2, lines 10-16; Figure 4; and Table 1.) The results show that the addition of increasing amounts of receptor antiserum corresponded to an increased inhibition of ^{35}S-labeled HRV binding to HeLa membranes. No inhibition of virus binding was observed with dilutions of control antiserum. (Tomassini thesis at 65, lines 4-8; and Tomassini article at 293, col. 2, line 16, to 294, col. 1, line 4.) Therefore, Tomassini's radio labeled HRV is capable of binding to membranes, demonstrating that the binding sites on radio labeled HRV are not denatured or disrupted by the radio labeling protocols, but are functional. Since radio labeled HRV binds membranes but does not bind to Tomassini's HRRP preparation, I believe that the most reasonable scientific explanation is that the binding sites on Tomassini's HRRP preparation are disrupted and not functional. Thus, in my opinion, the purification procedures taught in the Tomassini thesis and article disrupt the HRRP receptor structure and render it incapable of binding to HRV or LFA-1.

8. I note that the Examiner has further rejected the pending claims under 35 U.S.C. § 103(a) as allegedly being unpatentable over Tomassini, thesis 8624033 (1986) or Tomassini *et al.*, *J. Virol. 58*:290-295 (1986). (Office Action at page 5.) In making this rejection, the Examiner concluded that "the HRV receptor is ubiquitous in the human body, and thus one with ordinary skill in the art at the time of invention would have had a reasonable expectation of isolating HRRP (ICAM-1) from any tissue in the human body . . . ." *Id.* As I discussed *supra*, I

believe this conclusion is in error given the results disclosed in the Tomassini thesis and Tomassini article. Specifically, claim 71, from which claims 72-73 and 75-78 depend, is directed to a "purified or isolated ICAM-1 preparation . . . capable of binding to LFA-1, Mac-1, or p150,95. The authors of the Tomassini thesis and the Tomassini article indicate that they are unable to isolate a 90-kDa receptor protein that is capable of binding virus. Thus, I believe that one of ordinary skill in the art would have no reason to expect that Tomassini's or their ICAM-1 purification procedure would yield ICAM-1 capable of binding to HRV. Furthermore, in my opinion, based on the teachings of the Tomassini thesis and the Tomassini article, and the fact that the binding sites for LFA-1 and HRV overlap, one of ordinary skill in the art would thus have no reason to believe that their purification procedure would yield HRRP (ICAM-1) capable of binding to LFA-1, Mac-1, or p150,95.

9. I further note that the cDNA clones disclosed in the Tomassini thesis also would not express ICAM-1. On page 94 of the Tomassini thesis, a restriction map of several HRV receptor (ICAM-1) clones is depicted (clones 4A, 6, and 21). These clones can be distinguished from the clones described in the specification in several respects. First, clones 4A and 21 are significantly shorter than the full-length ICAM-1 gene. The ATG initiation codon should be located about 2,846 bases upstream of the polyadenylation site. Moreover, the ICAM-1 coding region is about 1,600 bp. *See* Specification at Figure 8. However, clone 4A is about 500 bp in length, while clone 21 is about 1,100 bp in length.

Second, the restriction site pattern of clones 4A, 21, and 6 is distinct from the restriction map obtained from the sequence of the actual ICAM-1 gene. There are no *Xba*I restriction sites in the ICAM-1 gene sequence. However, two *Xba*I sites are listed on the restriction map in the Tomassini thesis within about 2,800 bp upstream of the polyadenylation site, i.e., the region that should correspond to the putative ICAM-1 gene. There is only one *Hinc*II site in the coding region of ICAM-1, and the distances between the *Hinc*II site and a *Sac*I site should be about 905 base pairs and 1,884 base pairs based on the published gene sequence. The restriction map in the Tomassini thesis does not teach *Hinc*II and *Sac*I sites separated by these distances. Moreover, starting from the polyadenylation site, none of the restriction sites described in the Tomassini thesis match up with actual restriction sites determined from the gene sequence within a margin of error of 100 base pairs. This can be clearly seen in the attached figure (attached as Exhibit G), where the restriction map from the Tomassini thesis is aligned with the actual locations of the same restriction sites based on published sequence information.

These findings are strong evidence that the clones described in the Tomassini thesis represent a cloning artifact, or a fortuitous cross-reactivity of their anti-HRV-receptor antibody with another anti-ICAM-1 protein, and do not contain the ICAM-1 gene.

As further evidence that the Tomassini clones do not comprise the actual ICAM-1 gene, I note that a subsequent article published by the author of the thesis, Tomassini *et al.*, *Proc. Natl. Acad. Sci. 86*:4907-4911 (1989) (not prior art),

teaches the cloning of the ICAM-1 gene. A copy of this article is attached hereto as Exhibit H. To obtain the cloned gene, Tomassini *et al.* used a different cDNA library and different clones than the library and clones described in the thesis. In my opinion, if the clones described in the thesis actually contained the ICAM-1 gene, it would not have been necessary to clone the ICAM-1 gene from another source.

10. Finally, I note that the monoclonal antibody directed against the HRV receptor (ICAM-1) did not recognize the protein expressed from clone 4A, showing that the portion of ICAM-1 recognized by the antibody was not expressed in its native state. (Tomassini thesis at 85, lines 16-20.)

THAT, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this document or any patent associated herewith.

Oct 12, 2000

Date

Robert Rothlein